OFFICIAL

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Simantov, et al.

Serial No.: 09/730,379 Edna Gergel

Filed: December 5, 2000

For: THROMBOSPONDIN-BINDING REGION OF HISTIDINE-RICH GLYCOPROTEIN AND METHODS OF USE

Examiner: Misook Yu

Group Art Unit: 1642

Confirmation No: 7403

Docket: 955-7P CON

Dated: January 20, 2004

I hereby certify this correspondence is being sent by First Class Mail, postage prepaid and addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on January 20, 2004

Signature: _____

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF ROY L. SILVERSTEIN UNDER 37 C.F.R. §1.132

Sir:

I, Roy L. Silverstein, M.D., a co-inventor of the above-identified U.S. patent application, declares as follows:

1. I am Chief of the Division of Hematology, Medical Oncology and Stem Cell Transplantation at the Weill Medical College of Cornell University and New York-Presbyterian Hospital - Cornell Campus.

2. Experiments were conducted under my direct supervision and control for the purpose of demonstrating increased cutaneous angiogenesis and accelerated wound closure in transgenic mice expressing histidine-rich glycoprotein (HRGP). These experiments are described in Exhibit 1. Transgenic mice over-expressing HRGP were generated by transfecting normal mice with additional full length

2, 3, 26-45

murine HRGP genes driven by the keratin 14 (K14) promoter in basal keratinocytes (see exhibit 1, paragraph [0001] and figure 1). Two transgenic lines were established (Tg 1 and Tg 19). The Tg 1 line contained seven copies of the transgene and the Tg 19 line contained twelve copies. The skin extracts of the transgenic mice contained a greater than ten-fold increase in HRGP than control (wild-type) mice (see exhibit 1, paragraph [0002] and figure 2). Analysis of the blood vessel of whole mounts of the ears demonstrated an increase in vascular tortuosity and branching in the transgenic mice compared to control mice (see exhibit 1, paragraph [0003] and figure 3). Further, immunohistochemical staining of skin sections from wild-type and transgenic mice with an antibody against the endothelial cell marker PECAM (CD31), showed a greater number of CD31 positive cells in the transgenic mice than in the wild-type mice.

3. The observed increase in vascular tortuosity and branching, and the greater number of CD31 positive cells in the transgenic mice than in the wild-type control mice indicates that the increased expression of HRGP in the skin of transgenic mice leads to increased blood vessel formation (e.g., angiogenesis).

4. The two transgenic mouse lines (Tg 1 and Tg 19) were also utilized to examine the effect of increased expression of HRGP in a wound model *in vivo*. In one wound-healing model, the mice were subjected to full thickness punch wounds. The time to wound closure was measured. One of the transgenic mouse lines (Tg 1) showed accelerated wound closure compared to control mice without the HRGP transgene (see exhibit 1, paragraph [0005] and figure 4). The other transgenic mouse line (Tg 19) did not. The Tg 1 transgenic mouse line expressed more HRGP than both the Tg 19 transgenic mouse line and the control mice.

5. Another model of wound healing is also described in exhibit 1. The mice were implanted subcutaneously with polyvinyl alcohol sponges (see exhibit 1, paragraph [0006]). Analysis of the sponges demonstrated a greater amount of vascularization in the wound granulation tissue of the Tg 1 transgenic mouse line

than in the control mice (see exhibit 1, paragraph [0007] and figures 5 and 6). In addition, a greater number of fine fibrovascular networks was detected in the transgenic mice compared to the control mice (see exhibit 1, paragraph [0007] and figure 7).

6. Results similar to those for Tg 1 transgenic mouse line described in paragraph 5 above were also observed in the Tg 19 transgenic mouse line. Specifically, although to a lesser degree than that of the Tg 1 transgenic mouse line, analysis of the sponges also showed a greater amount of vascularization in the wound granulation tissue of the Tg 19 transgenic mouse line than in the control mice (see exhibit 1, paragraph [0008]).

7. The above results demonstrate that over-expression of HRGP promotes angiogenesis and promotes wound healing.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 1/21/04 Signature: _____
Roy L. Silverstein, M.D.